TRANSDUCTION AND TRANSFORMATION

By

M. L. Morse

Reprinted from
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 68, Article 2, Pages 324-334
October 21, 1957

Part II. Effects of Particles on Cells

TRANSDUCTION AND TRANSFORMATION*

By M. L. Morse

Colorado Foundation for Research in Tuberculosis, and the Department of Biophysics, University of Colorado, Denver, Colo., and the Department of Genetics, University of Wisconsin, Madison, Wis.

Genetically, transduction and transformation are similar processes; both are the transfer of fragments of the genotype of one cell to other cells. Both processes are important biologically because they can form a cell with a novel genotype. In addition, transduction and transformation can be a method of combining the genotypes of asexually reproducing cells. Both processes are of interest here because both are potentially an origin for neoplastic tissue.

Lederberg (1956b) has proposed that the term transduction be used for these processes in order to emphasize the similarity of the genetic consequences rather than the technical differences in performance. In this paper, transduction will be used as a generic term, and "phage transduction" and "DNA (deoxyribonucleic acid) transduction" will be used in a species sense. The information derived from study of the two processes has been different, however; while both have supplied information about the organization and function of genes, only DNA transduction has provided information on the chemical composition of the gene. This will continue to be the case, at least until the material within phage particles becomes available to manipulation and chemical analysis.

Phage-mediated transduction was first observed in *Salmonella* bacteria by Zinder and Lederberg (1952) and has been extensively studied in this group of microorganisms (Baron *et al.*, 1953; Iseki and Sakai, 1954; Uetake *et al.*, 1955; Zinder, 1954). It was found that a great variety of genetic markers was transduced by phage, including those for amino acid synthesis and carbohydrate fermentation, drug resistance, cell serotype, motility, and others. In fact, because most markers tested have been found to be transducible, it is believed that all the genes of *Salmonella* are subject to transfer by bacteriophage. Markers are usually transduced singly, however, indicating that only small fragments are subject to transfer. Two types of genetic markers can be conferred on a transformed cell by a transducing phage: (1) those that depend merely on the phage for transfer, whose subsequent expression is not dependent on the presence of phage in the clone; and (2) those that are inseparable from the vector of transduction. In the latter group the lysogenized (virus infected) cell is converted to a new geno-

* Paper No. 643 of the Department of Genetics, University of Wisconsin. The work at Madison was supported by Grant C 2157 from the National Cancer Institute, Public Health Service, Bethesda, Md.; from the National Science Foundation, Washington, D. C.; and from the Research Committee Graduate School, University of Wisconsin, with funds allocated by the Wisconsin Alumi Research Foundation, Madison, Wis.

type, a result comparable to the nontoxigenic to toxigenic conversion obtained by lysogeny in *Corynebacterium diphtheriae* (Groman, 1955). On the basis of these studies it was concluded that bacteriophage was the vector because of similarities in a number of properties between it and the vector of transduction.

The application of transduction as an analytic tool of the genetic material of *Salmonella* cells has been made in several laboratories (Stocker *et al.*, 1953; Demerec, *et al.*, 1955; Hartman, 1955). The results of these studies have shown that (1) some genes of *Salmonella* are sufficiently linked to be transduced simultaneously, and (2) many of these linked genes affect the same phenotype. The first of these findings made possible the mapping of some genes, and the sequences for genes involved in the synthesis of two amino acids, tryptophane and histidine, have been established. Not only was it found that the genes were closely linked, but the gene sequences corresponded to the sequences of enzymatic steps in the respective biosyntheses.

The recipient cell in transduction contains, momentarily at least, two different genes at a single locus; that is, it is heterogenotic for that locus. In the *Salmonella* transduction systems studied, the heterogenotic state does not persist longer than a few cell divisions, at most.

Phage-mediated transductions have been observed also between cells of *Escherichia coli* (Morse *et al.*, 1956a, 1956b; Lennox, 1955; Jacob, 1955) and between the "species" *Escherichia* and *Shigella* (Lennox, 1955). Several different phages were involved in these studies. In general, the frequency of transduction per phage particle was similar to that in *Salmonella*, about 1 per 10^5 to 10^7. As in *Salmonella*, linked transductions were observed infrequently, but the number of markers transduced at one time was, in some cases, larger than that observed in *Salmonella*. For example, Lennox observed the simultaneous transfer of four "unrelated" genes in *E. coli* K-12 by phage P1. Previously it had been shown that these four markers were linked by bacterial crosses. Both Lennox and Jacob found that the prophage state of one bacteriophage (lambda) could be transduced by another phage. An interesting feature of some of the clones studied by Lennox was the persistence of the transduced fragment which, in one case, was maintained through five serial single-colony transfers of the clone.

The phage lambda-*E. coli* K-12 system studied by Morse *et al.* (1956a, 1956b) has some features that distinguish it from the other phage-transduction systems.

It has been found that lambda, the bacteriophage for which most strains of *E. coli* K-12 are lysogenic, transduces only some genes that affect galactose fermentation (Gal genes). It is to these Gal genes that lambda is found to be linked in bacterial crosses and, presumably, is near (in the prophage form) on the K-12 chromosome. Further evidence that this spatial relationship exists comes from the observation that transducing lambda is found only when produced from lysogenic bacteria; that is, lambda grown from an exogenous source has not been found to possess that transducing activity which lambda from the "Gal-linked" state has.

The number of genes in *E. coli* K-12 affecting galactose fermentation is

large; at least seven have been studied thoroughly, and ten to twenty others less so.

The mutations to inability to ferment galactose that have been deeply studied have been designated as follows: Gal_1^-, Gal_2^-, . . . Gal_8^- (Morse *et al.*, 1956b). Some of the biochemical steps that these mutations affect have been identified (Kurahashi, 1956). The Gal_2^- and Gal_8^- mutations result in the loss of galactokinase activity; Gal_1^-, Gal_4^-, Gal_6^-, and Gal_7^- mutations cause loss of galactophosphate-uridyltransferase activity. These mutations have been distinguished as nonallelic by two tests: (1) bacterial crosses and (2) transductional comparisons. In the tests by the crossing method, galactose-positive recombinants were obtained in pairwise crosses between mutants. Tests by the transductional method also gave galactose-positive clones, that is, Gal_1^- (donor) ——× Gal_2^- (recipient) yielded galactose positives, but Gal_1^- ——× Gal_1^- did not, which also holds true for similar comparisons with the other mutants (Morse *et al.*, 1956b).

Both the positive and negative alleles of the Gal genes have been transduced by lambda. This can be seen most readily from a consideration of an example, the transduction from a Gal_1^- donor to a Gal_2^- recipient culture. The process may be diagrammed:

$$\underset{\text{(donor)}}{Gal_1^-Gal_2^+} \text{——}\times \underset{\text{(recipient)}}{Gal_1^+Gal_2^-} \text{——} \underset{\text{(heterogenote formed)}}{Gal_1^+Gal_2^-/_{ex}Gal_1^-Gal_2^+}$$

The heterogenote formed by lambda transduction, unlike heterogenotes in *Salmonella*, does not segregate to a haploid immediately, but persists and forms a clone that segregates about once per thousand cell divisions. Single gene heterogenotes ($^-/_{ex}^+$), as well as heterogenotes formed between mutants of the kinase group and transferase group, have a galactose-positive phenotype, that is, they ferment galactose. This indicates that under these conditions the positive alleles are dominant to the negative alleles.

The phenotypes of the Gal^- mutants and the corresponding single-gene heterogenotes on the indicator medium employed [eosin methylene blue (EMB) galactose agar] are shown in FIGURE 1. The dark colonies are fermenters and the white colonies are nonfermenters. Segregation in the heterogenotic clone is indicated by the presence of nonfermenters and mixed colonies. The segregation process is so rare that heterogenote clones can be maintained by transfer of the positive colonies.

The preparation of the stocks of the Gal^- mutants shown in FIGURE 1 will illustrate additional features of the lambda transduction system. In the example given above (Gal_1^- —× Gal_2^-), the heterogenote clone formed is galactose-positive and is selected from the large number of nontransformed galactose-negative cells on the indicator medium. With powerful lysates (to be described later) it is possible to make the transductions without selection, or in such a way that galactose-negative clones are produced from galactose-positive recipients. In the latter way the mutations Gal_1^- . . . Gal_7^- were transduced to the same galactose-positive clone. The stocks with these mutations shown in the figure, with the exception of the Gal genes, are isogenic. The isogenicity is reflected in the similarity of growth of these

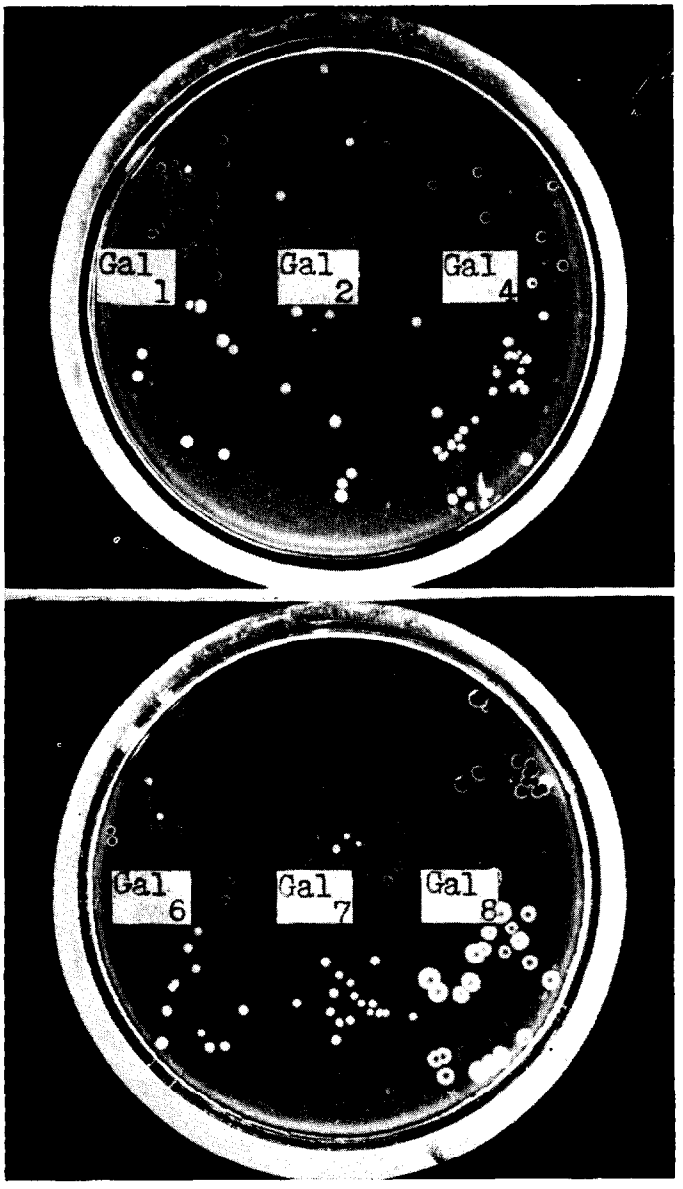

FIGURE 1. The galactose-negative mutants and their respective heterogenotes on EMB galactose agar. The dark colonies are galactose fermenters; the white colonies, galactose nonfermenters. In some cases the colonies are mixed, indicating segregation. The Gal$^-$ mutants are in the bottom row on each plate, and the corresponding heterogenotes ($-/_{ex}+$) are in the top rows. The Gal_1^-, Gal_2^-, Gal_4^-, Gal_6^-, and Gal_7^- cultures, with the exception of the Gal genes, are isogenic. Gal_8^- is not isogenic to the other stocks. This is reflected in a difference in colony size from the others. All cultures are prototrophic.

clones. The clone shown with the Gal_3^- mutation was not prepared in this way, is not isogenic with the others, and is reflected in a different growth habit.

As noted, heterogenotes formed between mutants of the kinase group and the transferase group are galactose-positive and resemble the single gene heterogenotes shown in FIGURE 1. Heterogenotes formed between mutants affecting the same enzymatic step (kinase or transferase), with one exception, have a galactose-positive phenotype when the positive alleles are in the *cis* position, but not when in the *trans* position. This indicates a positional effect for these loci. The $++/_{ex}--$ and $--/_{ex}++$ *cis* heterogenotes are galactose-positive; the $+-/_{ex}-+$ and $-+/_{ex}+-$ *trans* heterogenotes are galactose-negative (FIGURE 2). The galactose positives found in the *trans*-position clones are the result of a secondary process, crossing over, that forms the *cis* position ($++/_{ex}--$ or $--/_{ex}++$). The *trans*-position clones have been maintained by transferring colonies with a galactose-negative phenotype. Such clones have been shown to contain both alleles at each Gal locus.

The heterogenotes, whether galactose-positive or negative, segregate haploid progeny about once per thousand cell divisions. From a study of many separate segregations from a large number of heterogenotic clones, it has been possible to establish a pattern characteristic of most heterogenotes. The segregants from a heterogenote formed between a Gal^- donor and a nonallelic Gal^- recipient have the following Gal markers: (1) of the recipient cell, about 88 per cent; (2) of the fragment transduced from the donor culture, 10 to 11 per cent; and (3) of the combinations of markers from both donor and recipient, 1 to 2 per cent. This last class includes both galactose positives and double negatives, for example, $Gal_1^+Gal_2^+$ and $Gal_1^-Gal_2^-$, respectively. Such combinations indicate that crossing over is taking place in heterogenotic clones.

Further evidence for crossing over is found in the homogenotic segregants (for example, $Gal_1^-/_{ex}Gal_1^-$) obtained. Homogenotic segregants are, like the haploids, galactose-negative. Proof that they are homozygous-diploid for the Gal genes is obtained from galactose reversion studies. In contrast with haploid segregants and the parental Gal^- cultures, reversion to Gal^+ changes homogenotes to a heterogenotic condition, and segregation for galactose fermentation is observed. Homogenotic segregants of three types have been obtained from two locus heterogenotes and, presumably, if a sufficiently large sample were studied, the other types could be isolated The three types that have been observed are: (1) homogenotic for the Gal^- mutation of the recipient cell; (2) homogenotic for the Gal^- mutation of the donor cell; and (3) homogenotic for one mutation and heterogenotic for the other. The last type of homogenote indicates that crossing over has taken place at the multistrand stage.

Lambda produced from haploid cultures has low transducing activity; for example, 10^8 ultraviolet radiation- (UV-) induced cells yield 10^{10} phage particles, about 10^4 of which have transducing activity. These lysates give a low frequency of transduction with a sample of cells and are called LFT

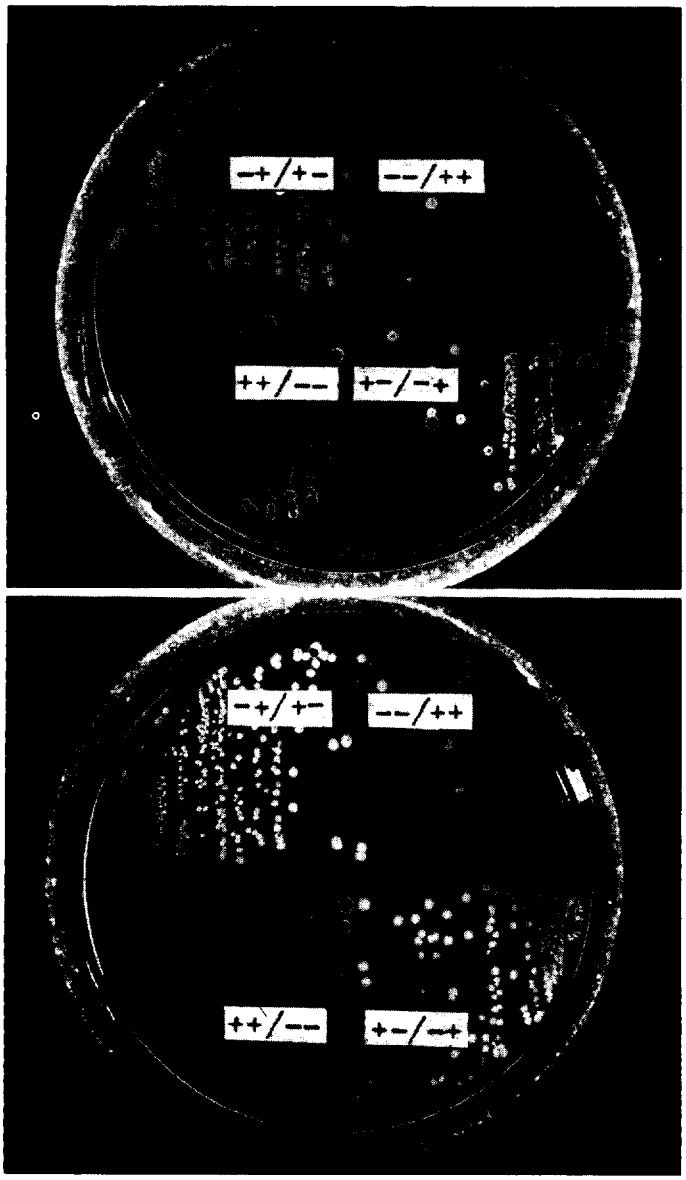

FIGURE 2. Heterogenotes, showing position effect. The formulae indicate the position of the genes; thus, $+-/-+$ is: genotype of the recipient/genotype of the fragment transduced. In the top plate are Gal_1^- and Gal_4^- heterogenotes; in the bottom plate, Gal_1^- and Gal_7^- heterogenotes. In each case the $++/--$, $--/++$ *cis*-position heterogenotes are galactose-positive; the $+-/-+$, $-+/+-$ *trans*-position heterogenotes are galactose-negative. The few galactose-positive colonies in the *trans*-position clones are the result of crossing over during previous growth to give *cis*-position recombinants.

lysates. In contrast with this, UV induction of 10^8 lysogenic (Lp^+), heterogenotic, or homogenotic cells produces about 10^8 lambda particles, nearly every one of which has transducing activity. When undiluted, lysates of the latter type give a high frequency of transduction (1 to 15 per cent of exposed cells) and are referred to as HFT lysates. It is the HFT lysates from homogenotic cultures that are used in Gal typing (Morse *et al.*, 1956b).

The low yield of lambda from heterogenotes and homogenotes suggests that the Gal genes have modified the multiplication of lambda. Further

TABLE 1
PRODUCTION OF TRANSDUCING LAMBDA IN HAPLOID AND HOMOGENOTIC CULTURES: RESPREADING EXPERIMENTS

Culture*	Number of cells induced to give transducing particles (spread plates)	Number of transducing particles released per induced cell (respread plates)
Haploid	697	574
	585	719
	704	746
	700	600
Mean	672	658
Homogenote	52	33
	30	35
	32	34
	43	
	50	
	56	
Mean	42	34

The donor cells in saline suspension were irradiated with UV and 0.1-ml. samples were plated on EMB galactose agar. After 2 hr. at 37° C., the indicated plates were respread with 0.1 ml. of broth. Under these conditions, lysis of the cells took place between 60 and 80 min. post UV. See text.

Survival of the UV-induced donors in the experiments were: haploid, about 15 per cent; homogenote, 0.5 per cent.

* The transductions were (diagrammatically): haploid, $F^- Gal_2^- \times F^- Gal_1^- Gal_4^- l.p^+$; homogenote, $F^+ Gal_2^-/_{ex} Gal_2^- \times F^+ Gal_1^- Lp^s$.

evidence for this modifying action is obtained from the study of the production of transducing lambda from haploid and heterogenotic and homogenotic cultures and from the study of the transductions produced by lambda from heterogenotic and homogenotic cultures.

The production of transducing lambda was investigated by respreading experiments; that is, a culture was UV-induced to form transducing phage, and samples were spread on a series of plates with an indicator culture. Time was allowed for lysis to take place, and then a number of the plates were respread. Comparison of the respread with the spread plates indicated

whether more than one transducing lambda particle was produced per lysing cell. In experiments with both haploid and heterogenotic cultures, spread and respread plates had equivalent numbers of transductions (TABLE 1). Thus, only one transducing phage was released per cell; the Gal genes had altered the multiplication of lambda from its usual course of yielding about one hundred particles per cell*.

In contrast to LFT transduction, transductions to lambda-sensitive recipients with HFT lysates yielded heterogenotic clones with two phenotypes: (1) those resistant to exogenous lambda and yielding lambda during subsequent growth [such clones being lysogenic (Lp^+) for lambda] and (2) those resistant to exogenous lambda but *not* yielding plaque-forming lambda during subsequent growth. In 58 heterogenotes studied, the relative frequencies of the two types was 45:13, respectively. The first phenotype is explained on the basis of lambda as vector of the transduction. The second can be explained on the basis of a lambda particle modified in some way by its association with the Gal genes. There are two observations that support this supposition: (1) that recipient cells of a mutant type incapable of absorbing lambda do not form heterogenotes of either phenotype and (2) that clones of the second type are resistant to exogenous lambda, suggesting a lambda specificity. One possible explanation is that these heterogenotes are lysogenized with a defective lambda particle, since haploid cultures lysogenized in this manner have been described previously (Lederberg and Lederberg, 1953). The heterogenotes in question, however, differ from lysogenic defectives (in addition to the heterogenotic condition for the Gal genes) in that they have not been found to yield plaque-forming lambda after UV induction, as do lysogenic defectives; also, they segregate lambda-sensitive progeny. On the supposition that these clones are a new type of lambda defective and, on the basis of the last observation, it is possible to give them the tentative genotype $Lp^{r/s}$. A corollary of this supposition is that they are diploid for lambda as well as for Gal genes. A comparison of lambda defectives (Lp^r) with $Lp^{r/s}$ heterogenotes is given in TABLE 2.

When $Lp^{r/s}$ heterogenotes segregate for galactose fermentation, they may segregate for lambda reaction. Thus, of 16 Gal- segregants from a heterogenote in which 2 Gal loci were segregating, 4 were lambda sensitive and 12 were of the parental heterogenote $Lp^{r/s}$ genotype. Gal-reversion studies on these segregants showed that the lambda sensitives were haploid, and the $Lp^{r/s}$ segregants (TABLE 3) were homogenotic ($Gal^-/_{ex}Gal^-$). Other $Lp^{r/s}$ heterogenotes have shown a similar segregational behavior. None have been found to form lambda defectives of the type previously reported in haploids.

If these transductions are the result of defective lambda particles, the defects in lambda are of two types: (1) a defect in the attachment of lambda

* Note (February 20, 1957): Experiments in other laboratories using a different lambda strain and different *E. coli* K-12 mutants have shown that the yield of transducing lambda is greater than one per cell. This indicates that, in some instances, lamdba multiplication may not be modified by Gal genes, and also that the Gal genes may multiply as lambda multiplies.

TABLE 2
COMPARISON OF $Lp^{r/s}$ HETEROGENOTES WITH LAMBDA-DEFECTIVE LYSOGENICS (Lp^r)

Cultures	Lambda plaques produced per 5×10^8 cells		Segregate Gal^- and/or lambda-sensitive cells
	Spontaneously	After UV induction	
1. Haploid lambda defectives			
W1924	0	2.8×10^4	no
W1027	0	7.0×10^2	no
W3172	0	1.5×10^4	no
2. $Lp^{r/s}$ heterogenotes			
W2341	0	0	yes
371a*	0	0	yes
371b*	0	0	yes

* About 10^6 to 10^7 cells were studied in these cases for lambda output. All $Lp^{r/s}$ segregated lambda-sensitive progeny.

TABLE 3
SEGREGATION FROM AN $Lp^{r/s}$ HETEROGENOTE*

Segregant phenotype	Number of segregants	Gal reversion studies	
		Reversions/segregant	No. reversions segregating
Gal_2^- lambda resistant	12	1	11
Gal_2^- lambda sensitive	3	1	0
Gal_4^- lambda sensitive	1	1	0

The heterogenote was resistant to lysis by exogenous lambda, and it segregated lambda sensitives and galactose negatives. Classification of the segregants is given. The reversion studies indicate that lambda-sensitive segregants are haploid; lambda-resistant segregants, homogenotic.

* The parent galactose-positive heterogenote was made:

$$\underset{\text{(donor)}}{Gal_4^+Gal_2^-} \times \underset{\substack{\text{Lambda sensitive}\\ \text{(recipient)}}}{Gal_4^-Gal_2^+} \rightarrow \underset{\text{(heterogenote)}}{Gal_4^-Gal_2^+/ex\ Gal_4^+\ Gal_2^-}$$

to the chromosome of *E. coli*, and (2) a defect such that lambda does not mature to plaque-forming particles.

In summary, it can be said that the association of lambda with the Gal genes has produced modifications of lambda behavior, both in the alteration of the multiplication in the vegetative state, and in the fact that incomplete or defective particles are formed.

DNA-mediated transductions (or transformations) have been described in a variety of bacterial species (Zamenhof, 1956; Ephrussi-Taylor, 1955;

Hotchkiss, 1954, 1956), but most of the investigations have been confined to the *Hemophilus* group and *Streptococcus pneumoniae* (pneumococcus). Although most of the transformations performed were for capsular type and other antigenic properties of the cells, other markers, such as resistance to drugs (streptomycin, penicillin, sulfonamide) and fermentation of carbohydrates (salicin and mannitol), have been transferred via DNA preparations. Most of these markers lack the technical advantages that feature markers employed in the study of phage-mediated transductions, and one of the improvements in DNA transformation studies will be in this area.

As in the phage-mediated transductions, most genetic markers are transferred singly by DNA preparations, but linked transductions have been observed. The frequency of transformation for a given marker under favorable circumstances has been on the order of a few per cent, but in many cases much lower frequencies have been obtained. The many factors that influence the success of transformation will be considered elsewhere in this monograph. The heterogenotes produced by DNA transformation, as in the case of most phage transductions, persist for only a few generations although, in a case of capsule-type transformation in *Hemophilus*, a clone "hybrid" or heterogenotic for two capsular markers was found to persist.

Experimentation with DNA-mediated gene transfers is largely directed towards two goals: (1) to find the minimum amount of DNA required to effect a transformation, and hence the molecular weight of a single gene; and (2) to fractionate DNA into genetic units so that chemical analysis of these units can be made. Some estimates of the required molecular weights have been made; they range from less than one-hundred thousand to several millions. The imprecision here is largely due to technical problems in the biological assay, a subject that will be dealt with by Ravin in the following paper. With regard to the separation of genes in DNA by chemical means, no progress appears to have been made. Apparently, chemical separational procedures have been insufficiently sensitive to accomplish the isolation of genetic units.

With regard to the "prospects for genetics of somatic and tumor cells," attention is called to a paper with this title by Lederberg presented at a conference on ascites tumors held by The New York Academy of Sciences (Lederberg, 1956a).

References

BARON, L. S., S. B. FORMAL & W. SPILMAN. 1953. Use of Vi phage lysate in genetic transfer. Proc. Soc. Exptl. Biol. Med. **83**: 292–295.

DEMEREC, M., I. BLOMSTAND & Z. E. DEMEREC. 1955. Evidence of complex loci in *Salmonella*. Proc. Natl. Acad. Sci. **41**: 359–364.

EPHRUSSI-TAYLOR, H. 1955. Current status of bacterial transformations. Advances in Virus Research. **3**: 275–307.

GROMAN, N. B. 1955. Evidence for the active role of bacteriophage in the conversion of nontoxigenic *Corynebacterium diphtheriae* to toxin production. J. Bacteriol. **69**: 9–15.

HARTMAN, P. 1955. Carnegie Inst. Wash. Year Book, **54**: 219.

HOTCHKISS, R. D. 1954. Bacterial transformation. J. Cellular Comp. Physiol. **45** (Suppl. 2): 1–14.

HOTCHKISS, R. D. 1956. The genetic organization of the deoxyribonucleate units func-

tioning in bacterial transformations. *In* Enzymes. Units of Biological Structure and Function. Academic Press. New York, N. Y.

ISEKI, S. & T. SAKAI. 1954. Transduction of biochemical properties in *Salmonella* E group. Proc. Japan. Acad. **30**: 143–147.

JACOB, F. 1955. Transduction of lysogeny in *Escherichia coli*. Virology. **1**: 207–220.

KURAHASHI, K. 1956. In press.

LEDERBERG, J. 1956a. Prospects for a genetics of somatic and tumor cells. Ann. N. Y. Acad. Sci. **63**(5): 662–665.

LEDERBERG, J. 1956b. Genetic transduction. Am. Scientist. **44**: 264–280.

LEDERBERG, E. M. & J. LEDERBERG. 1953. Genetic studies of lysogenicity in *Escherichia coli*. Genetics. **38**: 51–64.

LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage Pl. Virology. **1**: 190–206.

MORSE, M. L., E. M. LEDERBERG & J. LEDERBERG. 1956a. Transduction in *Escherichia coli* K-12. Genetics. **41**: 142–156.

MORSE, M. L., E. M. LEDERBERG & J. LEDERBERG. 1956b. Transductional heterogenotes in *Escherichia coli*. Genetics. **41**: 758–779.

STOCKER, B. A. D., N. ZINDER & J. LEDERBERG. 1953. Transduction of flagella characters in *Salmonella*. J. Gen. Microbiol. **9**: 410–433.

UETAKE, H., T. NAKAGAWA & T. AKIBA. 1955. The relationship of bacteriophage to antigenic changes in salmonellas of group E. J. Bacteriol. **69**: 571–579.

ZAMENHOF, S. 1956. Biology and biophysical properties of transforming principle. Prog. in Biophys. and Biophys. Chem. **6**: 85–120.

ZINDER, N. 1954. Bacterial transduction. J. Cellular Comp. Physiol. **45** (Suppl. 2): 23–49.

ZINDER, N. & J. LEDERBERG. 1952. Genetic exchange in *Salmonella*. J. Bacteriol. **64**: 679–699.